56. (Amended) An isolated recombinant animal adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence cleavable by a protease, said adenine nucleotide translocator polypeptide being separable from the fusion protein by cleavage with the protease, wherein the fusion protein is capable of localizing to a mitochondrial membrane and is capable of binding an ANT ligand .

REMARKS

Reconsideration of the present application in view of the following remarks is respectfully requested. Claims 42, 43 and 46-57 are pending. Claims 43, 49, 50, 54 and 55 have been canceled and claims 42, 46, 47, 51, 52 and 56 have been amended in a manner that more clearly defines certain subject matter encompassed by applicants' invention. Claim 46 has been amended solely to correct what would otherwise be an improper dependency on a canceled claim. Support for the amendments may be found in the specification, for example, at page 5, line 29 through page 6, line 13; at page 15, lines 3-10; at page 18, lines 4-24; at page 20, lines 4-30; at page 22, line 21 through page 23, line 11; at page 23, line 26 through page 24, line 8; at page 44, line 5 through page 45, line 17; at page 54, line 19 through page 57, line 27; and in the Examples (*e.g.*, pages 461-68, 74-88). Support for the amendments may also be found in the specification, for example, at page 26, line 28 through page 27, line 29; page 38, lines 12-13; page 82, lines 20-27; page 115, lines 1-11; page 116, line 1 through page 117, line 28 and page 118, line 29 through page 120, line 2. No new matter has been added.

The Office Action included a Notice of Draftsperson's Patent Drawing Review which objected to the Drawings as originally filed. Corrected drawings will be submitted after a Notice of Allowance has been received. Attached hereto is a marked-up version of the changes made to the claims by the current Amendment, the first page of which is captioned "Version with Markings to Show Changes Made."

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claim 42 stands rejected under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness deriving from its dependency on canceled claims. Applicants respectfully traverse this ground of rejection and submit that claim 42, as amended herewith, no longer depends from a canceled claim. Accordingly, applicants submit the rejection has been obviated and request that it be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

The Examiner rejected claims 42, 43 and 46-57 under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not adequately described in the specification. More specifically, the Action asserts that the specification fails to provide a representative number of species to describe the claimed genus of isolated adenine nucleotide translocator (ANT) polypeptides, and that structural and functional properties shared by the disclosed species, or by fragments and variants thereof, are not disclosed.

Applicants respectfully traverse these grounds for rejection and submit that the description of the claimed invention in the specification is sufficient to reasonably convey to a person having ordinary skill in the art that the applicants, at the time of filing the application, had possession of the claimed invention. The present invention is directed in part to an isolated recombinant human adenine nucleotide translocator polypeptide that localizes to a mitochondrial membrane, that is capable of binding an ANT ligand and that is produced by a method of culturing a host cell having a recombinant expression construct that has at least one regulated promoter operably linked to a nucleic acid encoding the adenine nucleotide translocator polypeptide. As known to the prior art and as described in the instant specification, over 30 adenine nucleotide translocator polypeptides are known (29 complete and 3 incomplete sequences) from a variety of organisms, several organisms encode two or three ANT isoforms, and the structure of this family of polypeptides is highly conserved (*see, e.g.*, specification, page 18, lines 22-27; and Fiore *et al.*, 1998 *Biochimie* 80:137, at page 138, column 2 under "Genomic Structure of the ADP/ATP Carriers" and at page 139, Figure 1). Further, there are a number of

functional properties associated with adenine nucleotide translocator polypeptides (Fiore *et al.*, page 138, column 1, last paragraph). Thus, within the genus of adenine nucleotide translocator polypeptides, it is well established that there is a high degree of structural and functional conservation among the species within the claimed genus. Accordingly, in view of the number of disclosed species of ANT polypeptides (*see, e.g.,* SEQ ID NOS:31-33), a person having ordinary skill in the art would recognize that applicants were in possession of the attributes common to the members of the genus.

Nevertheless, without acquiescing in the present rejection, but solely for purposes of advancing the prosecution of the instant application without prejudice to any related continuation, divisional, continuation-in-part, reissue or reexamination application, applicants note that as amended herein, the present invention is directed, according to certain presently claimed embodiments, to an isolated *human* adenine nucleotide translocator polypeptide that localizes to a mitochondrial membrane, that is capable of binding an ANT ligand and that is produced by a method of culturing a host cell having a recombinant expression construct that has at least one regulated promoter operably linked to a nucleic acid encoding the adenine nucleotide translocator polypeptide.

As noted in the specification, for example, at page 5, lines 1-8, and at page 14, lines 21-28, and as discussed in greater detail below, the present invention provides isolated human ANT polypeptides that are neither disclosed nor contemplated by the prior art where, *inter alia*, problems related to ANT solubility, toxicity, and/or a tendency to accumulate in inclusion bodies (*e.g.*, Miroux et al., 1996 *J. Mol. Biol.* 260:289, at pages 290-291 and Table 1) all precluded the preparation of the claimed ANT polypeptides with any reasonable expectation of success, even where the nucleic acid sequences of cDNAs encoding at least three human ANT isoforms have been known since 1989 (specification at page 18, lines 10-22). Further on this point, applicants note that an overexpressed ANT polypeptide that accumulates in inclusion bodies (*e.g.,* as described by Miroux et al.) is <u>not</u> an isolated recombinant ANT that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, as provided by the present invention. A person having ordinary skill in the art would readily appreciate from the present application that an overexpressed recombinant protein which forms inclusion bodies is not a properly folded polypeptide capable of localizing to a biological membrane such as a

mitochondrial membrane, and that inclusion bodies are not mitochondria. Such an ordinarily skilled artisan would also recognize that inclusion bodies are known to comprise denatured, concentrated and/or precipitated proteins which, by virtue of their improperly folded state, would be incapable of binding an ANT ligand. The Action fails to provide any evidence or reasoning that would lead a person having ordinary skill in the art to believe that the overexpressed proteins described by Miroux et al. have any of the attributes of the presently claimed invention, in particular, localization to a mitochondrial membrane and an ability to bind an ANT ligand.

Applicants respectfully submit that the instant specification abundantly teaches how to make and use a claimed human ANT polypeptide that is capable of binding an ANT ligand. For example, isolated recombinant human ANT polypeptides that are capable of binding ANT ligands, the ANT ligands themselves, and their use in ANT-binding assays are described at page 44, line 5 through page 45, line 13; and in the Examples (pages 61-122). ANT-mediated stoichiometric exchange of ATP and ADP across the inner mitochondrial membrane is described, for instance, at page 16, lines 17-23. As other examples, descriptions of ATP binding to an isolated human ANT3 polypeptide, and of how to make and use isolated human ANT3 polypeptides that are capable of binding to an ANT ligand, are also provided at pages 113-120 of the instant specification, including demonstration of ANT binding to ANT ligands that are atractyloside derivatives.

Thus, as disclosed in the specification and recited in the claims, the present application establishes human adenine nucleotide translocator polypeptides that are capable of binding to an ANT ligand. Accordingly, in view of the number of disclosed species of ANT polypeptides (*see*, *e.g.*, SEQ ID NOS:31-33), a person having ordinary skill in the art would recognize that applicants were in possession of the attributes common to the members of the genus. Additionally, applicants respectfully submit that the instant specification conveys to a person having ordinary skill in the art that the applicants had, at the time of filing, possession of isolated human ANT polypeptides, or variants or fragments thereof, which are capable of binding to an ANT ligand. As applicants have previously noted, the instant specification teaches that a "fragment" includes any ANT polypeptide that retains essentially the same biological function or activity as an ANT polypeptide (*e.g.*, specification, page 23, lines 3-7). Moreover, the biological functions or activities of ANT polypeptides or variants or fragments thereof are well known in

the art, such as binding ANT ligands (*see, e.g.*, specification, page 17, lines 23-30; and Fiore et al., page 138, column 1, last paragraph). As also discussed above, the instant specification teaches a person having ordinary skill in the art how to analyze human ANT polypeptides structurally (*e.g.*, specification, page 23, line 26 through page 24, line 17) and in functional assays of ANT ligand binding (*e.g.*, specification, Examples 12-14). Thus, based on the instant specification, a person having ordinary skill in the art (i) would recognize the presence of the claimed isolated human ANT polypeptide, (ii) could clearly identify the presence of a variant or fragment thereof, and (iii) would be able readily to determine whether such a polypeptide is capable of binding to an ANT ligand.

Applicants also respectfully submit that the instant specification clearly describes localization of an ANT polypeptide to a mitochondrial membrane, for example at page 26, line 28 through page 27, line 29; page 38, lines 12-13; page 82, lines 20-27; page 115, lines 1-11; page 116, line 1 through page 117, line 28 and page 118, line 29 through page 120, line 2. Moreover, the art well knows what is a mitochondrial membrane and how to isolate a mitochondrial subcellular fraction, and the cited passages of the instant application include examples describing in detail ANT ligand binding by isolated recombinant human ANT polypeptides that are components of mitochondrial membranes present in such fractions.

Therefore, applicants respectfully submit that the instant specification and claims adequately describe the claimed invention and, consequently, satisfies the requirements of 35 U.S.C. § 112, first paragraph. Accordingly, applicants respectfully request that this rejection be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 102(a)

The Examiner rejected claims 42, 43 and 46 under 35 U.S.C. § 102(a) as being anticipated by Marzo et al. (*Science 281*:2027-2031, 1998). More specifically, the Action asserts that Marzo et al. teach a purified human ANT2 protein. The Action alleges further that applicants' invention is the same product as that described by Marzo et al., but is merely derived via an alternative process.

Applicants respectfully traverse this ground for rejection. Applicants submit that the cited reference fails to meet every limitation of the instant claims (*i.e.*, claims 42 and 46, in view of the cancellation herewith of claim 43, which renders the rejection of claim 43 moot) and, therefore, Marzo et al. fail to anticipate the claimed invention. As disclosed in the specification and recited in the claims, the instant invention is directed in pertinent part to an isolated human adenine nucleotide translocator polypeptide that localizes to a mitochondrial membrane, that is capable of binding to an ANT ligand and that is produced by a method of culturing a host cell comprising a recombinant expression construct that has at least one regulated promoter operably linked to a nucleic acid encoding the adenine nucleotide translocator polypeptide.

Applicants respectfully submit that Marzo et al. fail to teach an isolated human adenine nucleotide translocator that is produced recombinantly, nor do Marzo et al. provide an isolated recombinant human ANT polypeptide that is capable of binding an ANT ligand, nor do Marzo et al. disclose an isolated recombinant human ANT polypeptide that localizes to a mitochondrial membrane.

Applicants respectfully submit that the Action misapplies the reference where it refers to Marzo et al. at page 2029, Column 1, lines 9-32, Fig. 2C and Fig. 4. A careful reading of Marzo et al. reveals that, contrary to the assertion in the Action that "Marzo et al. disclose a purified human ANT2 protein . . . that . . . was purified to greater than 95% homogeneity . . ." (Action, page 5, paragraph number 14), the ANT described in Fig. 2C is <u>not</u> a human ANT but is instead derived from rat brain (Marzo et al. at page 2029, Col. 1, line 15), which is therefore neither an isolated human ANT nor an isolated recombinant human ANT, and thus cannot be human ANT2 as alleged by the Action. Furthermore, the Action asserts that Marzo et al., at page 2029, Col. 1, lines 29-31, describe human ANT2 that was purified to greater than 95% homogeneity, but applicants submit that this is in fact a reference to the ANT shown in Fig. 4C,

as noted by Marzo et al. at page 2029, Col. 1, lines 32-37, wherein further the legend for Fig. 4C (Marzo et al., page 2030) clearly refers to "purified ANT from rat myocardium", which again is neither an isolated human ANT nor an isolated recombinant ANT, and thus cannot be human ANT2 as alleged by the Action. Figure 4A of Marzo et al. relates to a human ANT from an HT-29 cell, but applicants submit that the ANT of Fig. 4A is not a recombinant ANT, nor is the ANT described therein shown to be capable of binding an ANT ligand, nor is HT-29 a host cell comprising a recombinant expression construct comprising at least one regulated promoter operably linked to a nucleic acid encoding the ANT polypeptide. Applicants therefore submit that nowhere in Marzo et al. is a polypeptide meeting all the limitations of the presently claimed invention taught or even suggested.

Moreover with respect to recombinant methodologies, Marzo et al. merely describe recombinant expression of a 55 amino acid domain of human ANT2 in an intact yeast dihybrid cell system (Marzo et al., Figure 4B at p. 2030; note 22 at page 2031), and the teachings of Marzo et al. in this regard are limited to detection of protein-protein interactions within such intact cells. Applicants therefore respectfully submit that Marzo et al. fail to teach a recombinant, *isolated* human ANT polypeptide which, as disclosed in the instant specification and as recited in the instant claims, *localizes to a mitochondrial membrane* and is *capable of binding an ANT ligand.* The Action does not specifically point to any teaching by Marzo et al. that is directed to an isolated human ANT polypeptide that that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, wherein the ANT polypeptide is produced by a method comprising culturing a host cell comprising a recombinant expression construct comprising at least one regulated promoter operably linked to a nucleic acid encoding the adenine nucleotide translocator polypeptide.

Moreover, applicants are aware of no prior art disclosure relating to the subject matter of the instant claims (*i.e.,* a recombinantly produced human ANT polypeptide that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, as provided by the instant application). On this point, applicants respectfully submit that the *process* utilized to arrive at the instant polypeptide is novel, where an isolated recombinant human ANT polypeptide having the recited features is absent from the prior art. Therefore, applicants submit that the claimed product is not anticipated by the prior art.

Additionally, insofar as the cited reference is silent regarding whether the recombinant 55 amino acid human ANT2-derived polypeptide expressed in, but *not* isolated from, yeast, as disclosed by Marzo et al., is capable or incapable of binding an ANT ligand, applicants submit that the subject matter of the instant claims is patentably distinct from the product disclosed in the prior art. Applicants therefore submit that Marzo et al. fail to teach an isolated human ANT polypeptide that is capable of binding an ANT ligand and that is produced by culturing a host cell having a recombinant expression construct comprising a *regulated* promoter operably linked to a nucleic acid encoding the ANT polypeptide, as is provided by the present invention. Accordingly, applicants respectfully submit that the instant invention is readily distinguished over Marzo et al., and request that the rejection under 35 U.S.C. § 102(a) be withdrawn.

The Examiner also rejected claims 42 and 46 (and claim 43, which is canceled by the present amendment thus rendering its rejection moot) under 35 U.S.C. § 102(a) as being anticipated by Fiore et al. (*Biochimie 80*:137-150, 1998). More specifically, the Examiner asserts that Fiore *et al.* teach isolation and characterization of mitochondrial ADP/ATP carrier proteins (ANT proteins), providing evidence that ANT proteins are very well known in the art. In addition, the Examiner asserts that Fiore *et al.* disclose an amino acid sequence alignment of multiple sequences of known ANT proteins, particularly, human ANT1, ANT2 and ANT3 sequences.

Applicants respectfully traverse this ground for rejection and submit that Fiore et al. fail to anticipate the claimed invention. It is well settled that for a reference to anticipate a claim under 35 U. S. C. §102, the reference must teach every limitation of the claim. Applicants submit that Fiore et al. fail to provide every limitation of the claim, making application of this reference inapposite for rejection under §102. In the instant case, the Action concedes that Fiore et al. provide a review of ANT proteins. Applicants submit, however, that the teachings of Fiore et al. merely refer generally to isolation and characterization of ANT proteins from a variety of sources, but that Fiore et al. do <u>not</u> disclose the present invention. Moreover, Fiore *et al.* teach that the amino acid sequences of most ANT polypeptides described therein were deduced from nucleotide sequences (page 138, column 2, lines 1-3 under "Genomic Structure of the ADP/ATP Carriers") without disclosing which ANT polypeptides were isolated. In other words, Fiore et al.

nowhere expressly disclose actual isolation of a recombinant human ANT polypeptide according to the present invention.

In support of the rejection of claims 42 and 46, the Action points to Fiore et al. at page 138, first column, last four lines, but applicants submit that this passage refers to general properties of ANT polypeptides but fails to teach an isolated *human* ANT polypeptide as recited by the instant claims, and therefore the disclosure of Fiore et al. fails to meet the limitations of the instant claims. The Action also points to Fiore et al. at page 145, first column, lines 1-9, where, concededly, beef heart ANT is described. Here again, applicants submit that the Action has failed to point to an anticipating disclosure in the art, where the cited passage fails to provide an isolated *human* ANT polypeptide according to the present invention. By way of contrast, the present invention is directed in pertinent part to an isolated recombinant human ANT polypeptide that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, as discussed above. The Action nowhere points to any teaching or suggestion of the present invention in Fiore et al.; applicants therefore submit that the present invention is readily distinguished over the cited reference.

As described in the specification, for example, at page 21, lines 9-15, an "isolated" ANT polypeptide will include an ANT polypeptide that is removed from its original environment. Fiore et al. merely teach that known ANT polypeptide *sequences* (*i.e.*, the sequence information, but not any isolated human polypeptide) have been deduced from nucleotide sequences (page 138, column 2, lines 1-3 under "Genomic Structure of the ADP/ATP Carriers"), but Fiore et al. fail to disclose actual *isolation* of any human ANT polypeptides. Fiore et al. also fail to disclose *isolation* of a recombinant human ANT polypeptide that localizes to a mitochondrial membrane; Fiore et al. fail to disclose any *isolated* recombinant human ANT polypeptide that is capable of binding an ANT ligand; Fiore et al. fail to disclose any *isolated* recombinant human ANT polypeptide that is produced by culturing a host cell comprising a recombinant expression construct comprising a regulated promoter operably linked to an ANT-encoding nucleic acid. Applicants therefore submit that Fiore et al. fail to teach or suggest the subject matter of the instant claims.

Accordingly, applicants respectfully submit that the instant invention is readily distinguished over Fiore et al., and request that the rejection under 35 U.S.C. § 102(a) be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 103(a)

The Examiner rejected claims 42, 43 and 46-57 under 35 U.S.C. § 103(a) as being unpatentable over Fiore et al. (*Biochimie 80*:137-150, 1998) in view of Rosenberg (*Protein Analysis and Purification: Benchtop Techniques*, Birkhäuser, Boston, pp. 335-347, 1996). In particular, the Examiner alleges that Fiore *et al.* teach a yeast ANT fusion protein having a polyhistidine tag, and that Rosenberg describes fusion proteins comprising a protein of interest fused to a marker enzyme or an affinity tag. The Action then asserts that a person having ordinary skill in the art would have found it obvious to express human ANT as an epitope tagged fusion protein for ease of detection and/or ligand affinity purification, by substituting a human or animal ANT sequence for the yeast ANT sequence of Fiore et al.

The Examiner also rejected claims 42, 43, 50 and 52-55 under 35 U.S.C. § 103(a) as being unpatentable over Adrian *et al.* (*Molecular and Cellular Biology 6*(2):626-634, 1986), in view of Fiore *et al.* The examiner alleges that Adrian *et al.* describe amino acid sequence requirements for mitochondrial localization of a yeast ANT fusion protein comprising an enzyme reporter molecule, β-galactosidase. The Action then asserts that it would have been obvious to substitute human ANT provided by the teachings of Fiore *et al.* for yeast ANT according to Adrian *et al.*, to express human ANT as a β-galactosidase fusion protein. The Examiner further cites Brandolin *et al.* (*Biochemistry 24*: 1991-1997, 1985), a publication cited by Fiore *et al.* (*supra*), as allegedly providing further evidence that isolation of ANT proteins was well known to the art at the time of filing the instant application.

Applicants respectfully traverse these grounds for rejection. The cited references, alone or in combination, fail to teach or suggest an isolated recombinant human adenine nucleotide translocator polypeptide that localizes to a mitochondrial membrane and is capable of binding an ANT ligand, or an isolated human or animal adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide, which is capable of localizing to a mitochondrial membrane and binding an ANT ligand, fused to at least one additional polypeptide

sequence. As noted above, Fiore et al. disclose several ANT polypeptide *sequences* that have been deduced from nucleotide sequences, but Fiore et al. fail to teach or suggest actual isolation of any *human* ANT polypeptide, or of any human or animal ANT *fusion proteins*. For reasons provided herein, none of the other references cited in the Action remedy these deficiencies of Fiore et al. As discussed in greater detail below, applicants submit further that the present invention is nonobvious when secondary factors, and in particular the identification of a long-felt need and the failure of others, are considered.

As noted in the Action, Fiore *et al.* describe a yeast strain expressing a yeast ANT fusion protein carrying a polyhistidine tag at the C-terminus, which was constructed to allow purification by immobilized metal ion affinity chromatography. According to the Action, however, Fiore *et al.* concededly fail to teach human or animal ANT fusion polypeptides. Contrary to the assertion in the Action, the addition of Rosenberg fails to remedy the deficiencies of Fiore *et al.* Rosenberg teaches protein fusions between a protein of interest and an enzyme or affinity tag, but of the myriad possible proteins of interest that could be fused to an enzyme or affinity tag of Rosenberg, nothing in the cited references would have motivated a person having ordinary skill in the art to combine the fusion protein tagging method of Rosenberg with a human or animal ANT polypeptide that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, w*ith a reasonable expectation of success*. In particular, and for reasons elaborated herein, recombinant human and animal ANT expression as known to the prior art had failed to overcome the technical problem of recombinant ANT that localized only to inclusion bodies (*e.g.*, Miroux et al., discussed above; see also discussion of Miroux et al., *infra*), in which form the recombinant ANT is <u>not</u> localized to a mitochondrial membrane and in which form the recombinant ANT is <u>not</u> capable of binding an ANT ligand.

Rosenberg teaches the use of β-gal as a fusion partner in the construction of a fusion protein, and the use of an engineered protease cleavage site between the fusion partner and the protein sequence of interest in a fusion protein, in order that they may be separated during purification. The teaching of Rosenberg, however, is merely cumulative subject matter, as the instant specification discloses several fusion enzymes and affinity tag sequences that are known in the art (see, *e.g.*, specification page 21, line 20 through page 24, line 14; Examples 1, 2, and 3). Furthermore, Rosenberg is a general reference regarding the construction and use of fusion

proteins and, therefore, provides no teaching or suggestion pertaining to the claimed isolated adenine nucleotide translocator polypeptides and fusion polypeptides. Thus, the combined cited prior art does not render the claimed invention obvious, where it is well settled that in order for prior art to be modified in a manner supporting a *prima facie* case of obviousness, the prior art must suggest the *desirability* of making such a modification. *In re Fritch*, 922 F.2d 1260; 23 USPQ2D 1780 (Fed. Cir. 1992).

The Action fails to provide specific reasoning in support of the assertion that the present invention would have been obvious at the time of filing the instant application, in view of the references cited by the Examiner and given the level of ordinary skill in the art. By way of contrast, applicants submit that if anything, the state of the art pointed away from arriving at the present invention with any reasonable expectation of success. For example, based on the teachings of Miroux et al. (1996 *J. Mol. Biol.* 260:289), which is also discussed above and a copy of which is enclosed for the Examiner's convenience, applicants submit that a person having ordinary skill in the art would have understood that recombinant expression of an ANT polypeptide is hardly a routine matter.

More specifically, Miroux et al. describe efforts to express various recombinant proteins, including mammalian ANT, in a bacterial expression system. Multiple problems are described with regard to efforts to express recombinant ANT, including toxicity to host cells, poor solubility of the recombinant product and accumulation of recombinant ANT in inclusion bodies (*e.g.*, Miroux et al., 1996 *J. Mol. Biol.* 260:289, at pages 290-291 and Table 1), which applicants submit would be recognized by those familiar with the art as a form amenable neither to ready isolation nor to functional binding interactions with an ANT ligand. Thus, and contrary to the assertion in the Action, applicants submit that it would not have been obvious to express well known ANT sequences to arrive at an isolated polypeptide or fusion protein having the recited features of the claimed invention, nor could the skilled artisan reasonably expect to succeed in doing so, given the problems that plagued the art as exemplified in Miroux et al. Applicants therefore respectfully submit that it would be misguided to believe that the person having ordinary skill in the art at the time of the present application knew, with a reasonable expectation of success, how to arrive at the instant invention.

Applicants also respectfully submit that Adrian et al. in view of Fiore et al. would not render the present invention obvious. The disclosure of Adrian et al. is limited to a determination of whether yeast ANT shares mitochondrial targeting sequence motifs with other typical mitochondrial proteins, but Adrian et al. fail to contemplate in any way the recombinant expression of human ANT polypeptides that are capable of binding to an ANT ligand, or of human or animal ANT fusion proteins, according to the present invention. The Action alleges that a person having ordinary skill in the art would have found it obvious to substitute human or animal ANT instead of the disclosed yeast ANT in order to study mitochondrial localization sequences in human or animal ANT, but applicants respectfully submit that such an allegation is beside the point, where Adrian et al. are concerned only with subcellular localization targeting motifs while the present invention is not so limited. The Action further points to Brandolin et al. (1985 *Biochem.* 24:1991) as evidence that isolated animal ANTs were well known to the art, but applicants submit that this reference, alone or in combination with any other cited art, fails to teach or suggest the present invention. Applicants therefore respectfully submit that reference to Brandolin et al. is beside the point, where the beef heart ANT of Brandolin et al. is neither a recombinant human ANT according to the instant claims, nor is it a human or animal ANT fusion protein as provided by the instant application.

Furthermore, applicants submit that the cited references alone or in combination fail to suggest that recombinant ANT expression could be achieved *with a reasonable expectation of success* if human or animal ANT sequences were substituted for the yeast sequences of Adrian et al. Thus, where the prior art failed to suggest to the person having ordinary skill in the art that the presently claimed ANT polypeptides should be made according to the present invention, and where, for reasons discussed herein, such a skilled artisan would not have been provided with a reasonable expectation of success in doing so based on the prior art, applicants submit that *prima facie* obviousness has not been established. *See, e.g., In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Applicants also respectfully submit that the present invention is nonobvious when "secondary" factors, and in particular the identification of a long-felt need and the failure of others, are considered. It is well established that considerations such as long-felt but unsolved needs, and the failure of others to arrive at applicants' invention, are not only relevant to the

obviousness inquiry, but must be considered when present. *Custom Accessories Inc., v. Jeffrey-Allan Industries Inc.,* 807 F.2d 955; 1 USPQ2d 1196 (Fed. Cir. 1986); *Ryko Manufacturing Co. v. Nu-Star Inc.*, 950 F.2d 714, 21 USPQ2d 1053, 1057 (Fed. Cir. 1991).

Hence, and as noted above, applicants respectfully submit that where cDNA sequences encoding a human ANT polypeptide were known as early as 1987, and where recombinant protein expression methods were established well before 1987, a long-felt need for reliable expression of human ANT polypeptides was present at the time of filing the instant application in 1998. On this point, applicants note that prior to the present invention, and despite the fact that a human ANT cDNA sequence had been known for over ten years, at the time of filing there had been no successful expression and isolation of a recombinant human ANT polypeptide that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, and the Examiner has failed to provide any evidence for same. For reasons discussed herein, the overexpression of ANT as a non-functional product that accumulates in inclusion bodies as described by Miroux et al. did not lead to an ANT polypeptide having the attributes of the presently claimed invention.

In addition, the attention directed to human and animal ANT polypeptides by numerous investigators, as evidenced by the prior art references cited throughout the instant specification (*e.g.,* page 18, lines 5-22; pages 44-45; Fiore et al.; and elsewhere, which references do not disclose recombinant ANT and which further do not disclose recombinant human ANT) makes clear the recognition in the art of a compelling need for a consistent, readily-produced and reliable source of such ANT polypeptides. Applicants submit that it is widely appreciated in the biotechnology art that recombinant protein production methods offer numerous advantages over non-recombinant methods, and that there was an unmet art-established need for recombinant human ANT that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand. Moreover, and as stated above, applicants are unaware of any successful production by others of any isolated recombinant human ANT polypeptide, and certainly not of an isolated recombinant human ANT polypeptide that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand, or of isolated ANT fusion proteins, according to the instant invention. In view of the absence of any such disclosures from the prior art, and further in view of unsuccessful efforts to express recombinant ANT in a useful form (*e.g.,* Miroux et al., *supra*),

applicants therefore respectfully submit that the present invention is nonobvious when such secondary considerations are taken into account.

Applicants therefore respectfully submit that the Action has not set forth a *prima facie* case of obviousness. As discussed above, the cited references fail to provide a suggestion or motivation for a person having ordinary skill in the art to modify or combine the prior art teachings to arrive at the claimed invention with a reasonable expectation of success, and secondary considerations clearly show the invention to be non-obvious. Accordingly, applicants respectfully request that this rejection be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 101

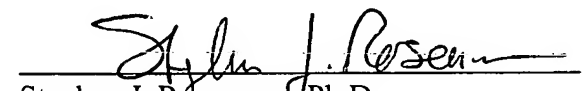
The Examiner also provisionally rejected claims 42, 43 and 46-57 under 35 U.S.C. § 101, for alleged double patenting in view of claims 42, 43 and 46-57 of co-pending Application No. 09/185,904.

Applicants respectfully traverse this rejection. The rejections of claims 43, 49, 50, 54 and 55 are obviated by the cancellation of these claims according to the amendment submitted herewith. With regard to the remaining instant claims 42, 46-48, 51-53 and 56-57, applicants submit that the provisional double patenting rejection has been rendered moot by the present amendment, according to which the allegedly conflicting claims are no longer coextensive in scope. As this is a provisional rejection, at such time as the instant claims are in otherwise allowable condition, applicants reserve the right to further amend and/or to cancel one or more claims in the present application and/or in co-pending Application No. 09/185,904 without prejudice to either application or to any related continuation, divisional, continuation-in-part, reissue or reexamination application.

All of the claims remaining in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited. If the Examiner does not believe the claims are allowable for any reason, the Examiner is encouraged to telephone the undersigned at (206) 622-4900.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC



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SJR:kw

Enclosure:
Copy of Miroux et al. (1996 *J. Mol. Biol.* 260:289)

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Version with Markings to Show Changes Made

42. (Amended) An [ANT] isolated recombinant human adenine nucleotide translocator polypeptide that localizes to a mitochondrial membrane and that is capable of binding an ANT ligand and that is produced by [the] a method [of any one of claims 39-41] comprising culturing a host cell comprising a recombinant expression construct comprising at least one regulated promoter operably linked to a nucleic acid encoding the adenine nucleotide translocator polypeptide.

46. (Amended) The isolated polypeptide of claim 42 [43] wherein the human adenine nucleotide translocator polypeptide is recombinant ANT3 or a variant or fragment thereof.

47. (Amended) An isolated recombinant human adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence, wherein the fusion protein localizes to a mitochondrial membrane and is capable of binding an ANT ligand.

51. (Amended) An isolated human adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence cleavable by a protease, said adenine nucleotide translocator polypeptide being capable of localizing to a mitochondrial membrane and capable of binding an ANT ligand and separable from the fusion protein by cleavage with the protease.

52. (Amended) An isolated adenine nucleotide translocator fusion protein comprising a first polypeptide that is an animal adenine translocator polypeptide that is capable of localizing to a mitochondrial membrane and capable of binding an ANT ligand and that is fused to at least one additional polypeptide sequence.

56. (Amended) An isolated recombinant animal adenine nucleotide translocator fusion protein comprising an adenine translocator polypeptide fused to at least one additional polypeptide sequence cleavable by a protease, said adenine nucleotide translocator polypeptide being separable from the fusion protein by cleavage with the protease, wherein the fusion protein is capable of localizing to a mitochondrial membrane and is capable of binding an ANT ligand .